## REMARKS

### I. Status of the Application

Claims 1-46 are presently pending in the application. Applicants gratefully acknowledge the Examiner's acceptance of the drawings filed on August 9, 2000. Claims 6-8, 41, 43, 45 and 46 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Claims 1, 3, 4, 6-13, 23, 24, 27, 29, 32, 33 and 36-41 stand rejected under 35 U.S.C. §102(a) as being anticipated by Carulli et al. (1998) *J. of Cellular Biochemistry Supplements* Vol. 30/31, pages 286-296. Claims 5, 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Carulli et al. in view of Lockhart et al., WO 97/10365. Claims 2 and 3 stand rejected as being unpatentable over Carulli et al. in view of Serafini et al., U.S. Patent No. 6,110,711, and Van Gelder et al. U.S. Patent No. 6,291,170.

Applicants have amended the claims to more clearly define and distinctly characterize Applicants' novel invention. Support for the amendments can be found in the specification and the claims as originally filed. Specifically, support for the amendments to claim 43 to recite "gene expression levels," can be found in the specification at least at page 1, line 31 to page 2, line 1, where Applicants teach changes in the expression levels of particular genes. Claim 25 was amended to address formal matters. Claims 6-8 and 41 were amended to correct dependencies. Claim 46 was amended to recite active method steps.

Applicants respectfully request entry and consideration of the foregoing remarks, which are intended to place this case in condition for allowance. Applicants respectfully submit that the amendments presented herein do not raise new issues requiring further search.

## II. Objections

At page 2, paragraph 2 of the instant Office Action, claim 25 stands objected to under 37 C.F.R. §1.75(c), as being of improper dependent form for failing to further limit the subject matter of the previous claim. The Examiner states that claim 25 is dependent on claim 2, which employs RNAs isolated from at least one cell, but that claim 25 recites a further step of cleaving "nucleic acids" into fragments, broadening claim 1, from using an RNA to more generic "nucleic acids." The Examiner requires Applicant to cancel the claim or amend the claim to place it in proper dependent form, or to rewrite the claim in independent form.

Without acquiescing to the objection, claim 25 was amended to remove the language "nucleic acids" and add the language "the population of RNA," thus obviating this objection.

## II. Claims 6-8, 41, 43, 45 and 46 are Definite

At page 2, paragraph 3 of the instant Office Action, claims 6-8, 41, 43, 45 and 46 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants respectfully traverse these rejections in view of the amended claims now presented.

The Examiner is of the opinion that claims 6-8 and 41 recite the limitation "the conditions of incomplete extension," but their parent claim does not recite any step regarding incomplete extension, rendering the claims lacking in antecedent basis, and that claims 45 and 46 are indefinite by way of their dependency on claim 41. In response, Applicants have amended claims 6-8 and 41 to depend from claim 4, which recites "incomplete extension," thus rendering this rejection moot.

The Examiner is of the opinion that claim 43 is indefinite for reciting the phrase "determining the *nature* and function of a plurality of single cells," because it is unclear what metes and bounds are covered by the step determining "the nature" of single cells. Without acquiescing to this rejection, claim 43 was amended to replace "the nature" with "gene expression levels," thus rendering this rejection moot.

The Examiner is of the opinion that claim 46 is indefinite for reciting the phrase "the plurality of cells can be deduced to have stem cell potentials" because the claim does not have any active steps. The Examiner states that the claim merely recites an intended use without any active limitation. Without acquiescing to this rejection, claim 46 was amended to recite the active method step of "deducing whether" one single cell from the plurality of cells has stem cell potential, thus obviating this rejection.

Accordingly, Applicants respectfully request that the rejection of claims 6-8, 41, 43, 45 and 46 under 35 U.S.C. §112, second paragraph be reconsidered and withdrawn.

## III. Claims 1, 3, 4, 6-13, 23, 24, 27, 29, 32, 33 and 36-41 are Novel over Carulli et al.

At page 3, paragraph 6 of the instant Office Action, claims 1, 3, 4, 6-13, 23, 24, 27, 29, 32, 33 and 36-41 stand rejected under 35 U.S.C. §102(a) as being anticipated by Carulli et al. (1998) *J. of Cell. Biochem. Suppl.* 30/31:286. The Examiner is of the opinion that Carulli et al. anticipates the invention as claimed. Applicants respectfully traverse this rejection.

The pending claims are directed to novel methods of monitoring gene expression wherein RNA is obtained from a sample ***comprising fewer than 1000 cells*** **or** ***from a single cell***, a first population of cDNA is generated from the RNA, the first population of ***cDNA*** is ***linearly amplified*** to produce a linearly amplified second population of cDNA, the linearly amplified

second population of cDNA is labeled with a detectable label, and an array of probes is contacted with the linearly amplified population of labeled cDNA to determine relative expression of at least two genes. Applicants' claimed invention has been demonstrated to result in linear amplification of cDNA, to exhibit an excellent correlation to actual gene expression in the cell, and to represent genes having low levels of expression in the cell (specification, page 32, line 31 to page 35, line 2; Tietjen et al. (2003) Neuron 38:161, given to Examiner Siew at January 20, 2004 interview).

Carulli et al. fails to anticipate the claimed invention. Applicants respectfully submit that for a reference to anticipate a claim, the reference must teach each and every element of the claim. Nowhere does Carulli et al. teach or suggest obtaining RNA from a sample comprising fewer than 1000 cells or from a single cell. In fact, the Examiner admits "Carulli et al. do not explicitly disclose the number of cells employed in obtaining the population of RNAs" (Office Action, page 6) and "Carulli et al. do not disclose the method involving the use of a single cell for extracting RNA population...nor a method involving obtaining a single cell population of RNA from each cell of a plurality of cells" (Office Action, page 10).

The Examiner also states, however, that Carulli et al. disclose a method of determining differential expression of two or more genes in one or more cells (Office Action, page 3). Applicants respectfully disagree. Carulli et al. does not teach obtaining RNA from a single cell or from a sample comprising fewer than 1000 cells, as claimed by Applicants. Further, Carulli et al. fails to recognize that this would even be possible. In fact, using the methods taught by Carulli et al., one of skill in the art would not be able to practice the claimed invention. Carulli et al. teaches deriving RNA from tissue or cell samples, generating a labeled cDNA pool from the RNA by reverse transcription, and then hybridizing the cDNA pool to a microarray (page

290, left column first full paragraph). If such a sample was obtained from fewer than 1000 cells or from a single cell, one would not be able to detect the labeled cDNA on an array because there simply would not be a sufficient level of hybridized cDNA for detection.

The Examiner is also of the opinion that Carulli et al. teaches that ***cDNA amplification*** is achieved via linear amplification (Office Action, page 4). Applicants respectfully submit that this is not so. Not only does Carulli et al. fail to teach or suggest a linearly amplified population of labeled cDNA as claimed by Applicant, but Carulli et al. actually ***teaches away from amplification of cDNA***. Carulli et all states, "protocols that use PCR ***amplification of the cDNA***, have typically resulted in ***inconsistent amplification*** of the independent messages in the cell or tissue sample" (page 290, left column, second paragraph, emphasis added). Given that Carulli et al. teaches that cDNA amplification is inconsistent, one of skill in the art would not be motivated to attempt linear amplification of cDNA based on the teachings of Carulli et al.

Although Carulli et al. speaks to linear amplification protocols that may amplify transcripts uniformly, these protocols are not directed to the amplification of cDNA, as required by the instant claims, but are instead directed to the generation of labeled ***antisense RNA*** using a ***single round*** of *in vitro* transcription (Lockhart et al. (1996) *Nature Biotech.* 14:1678, left column, first full paragraph). Applicants have discovered that the amount of RNA generated from less than 1000 cells using one or even two rounds of RNA amplification would not be detectable using microarray analysis, and that a third round of amplification would result in ***non-linear*** amplification of the starting RNA population (Amendment and Response mailed February 6, 2004, interview with Examiner Siew, January 20, 2004). Thus, Carulli et al., alone or in combination with Lockhart et al., fails to teach or suggest linearly amplified cDNA, as required by the pending claims.

Accordingly, Carulli et al. fails to teach or suggest each and every element of the claimed invention. Thus, Applicants respectfully request that the rejection of claims 1, 3, 4, 6-13, 23, 24, 27, 29, 32, 33 and 36-41 under 35 U.S.C. §102(a) as being anticipated by Carulli et al. be reconsidered and withdrawn.

## IV. Claims 5, 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 are Nonobvious over Carulli et al. in view of Lockhart et al.

At page 6, paragraph 2 of the instant Office Action, claims 5, 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Carulli et al. in view of Lockhart et al., WO 97/10365. The Examiner is of the opinion that the claimed invention is *prima facie* obvious over the cited references. Applicants respectfully traverse this rejection.

As discussed above, Carulli et al. fails to teach or suggest each and every element of Applicants' claimed invention. Lockhart et al. fails to cure the deficiencies of Carulli et al. The Examiner is of the opinion that absent evidence to the contrary, a fine needle biopsy as taught by Lockhart et al. "would necessarily have less than 100 cells." However, nowhere does Lockhart et al. teach or suggest a ***linearly amplified population of labeled cDNA*** obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims.

Thus, the combination of references fails to teach or suggest the claimed invention. Accordingly, Applicants respectfully request that the rejection of claims 5, 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 under 35 U.S.C. §103(a) be reconsidered and withdrawn.

## V. Claims 2 and 43 are Nonobvious over Carulli et al. in view of Serafini et al. and Van Gelder et al.

At page 10, paragraph 1 of the instant Office Action, claims 2 and 43 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Carulli et al. in view of Serafini et al., U.S. Patent No. 6,110,711, in light of Van Gelder et al., U.S. Patent No. 6,291,170. The Examiner is of the opinion that the claimed invention is *prima facie* obvious over the cited references. Applicants respectfully traverse this rejection.

As discussed above, Carulli et al. fails to teach or suggest each and every element of Applicants' claimed invention. Serafini et al. fails to cure the deficiencies of Carulli et al. Serafini et al. neither teaches nor suggests a ***linearly amplified population of labeled cDNA*** obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims. The Examiner is of the opinion that Serafini et al. teaches amplifying the mRNA of a single cell, and that Serafini et al. employs a linear amplification process at column 3, line 7. Applicants respectfully submit that Serafini et al. does not teach a linearly amplified population of ***cDNA***, as claimed by Applicants. Instead, Serafini et al. teaches that "***mRNA*** may be amplified" (column 3, line 5, emphasis added). Serafini et al. teaches that mRNA may be used to form cDNA, and that the cDNA may be repeatedly transcribed to form a plurality of amplified RNA (column 6, line 29 to column 8, line 16). Applicants have discussed above that amplified RNA generated from less than 1000 cells using one or even two rounds of RNA amplification would not be detectable using microarray analysis, and that a third round of amplification would result in ***non-linear*** amplification of the starting RNA population. Accordingly, one of skill in the art could not practice the claimed methods using the teachings of Serafini et al., alone or in combination with Carulli et al.

Van Gelder et al. fails to cure the deficiencies of Carulli et al. and Serafini et al. Van Gelder et al. neither teaches nor suggests a ***linearly amplified population of labeled cDNA*** obtained from fewer than 1000 cells for use in determining relative expression of at least two genes, as required by the instant claims. Instead, Van Gelder et al., like Serafini et al., is directed to transcribing amplified RNA from cDNA. For at least the reasons set forth above for Serafini et al., the teachings of Van Gelder et al., alone or in combination with the primary references, fails to teach or suggest Applicants' claimed methods.

Thus, the combination of references fails to teach or suggest the claimed invention. Accordingly, Applicants respectfully request that the rejection of claims 2 and 43 under 35 U.S.C. §103(a) be reconsidered and withdrawn.

## VI. Conclusion

Having addressed all outstanding issues, Applicants respectfully request entry and consideration of the foregoing amendments and reconsideration and allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is requested to telephone the undersigned at the number below.

Respectfully submitted,

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